PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY
(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION See Form PCT/IPEA/416	
696655-FA		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/SE2002/001903	18.10.2002	
International Patent Classification (IPC) or national classification and IPC		
A23K 1/00, A61K 35/74, C12R 1/225, A23L 1/03		
Applicant		
Biogaia AB et al		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 5 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *(sent to the applicant and to the International Bureau)* a total of 2 sheets, as follows:
      ☒ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s))
      _____, containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).
4. This report contains indications relating to the following items:
   - ☒ Box No. I Basis of the report
   - ☐ Box No. II Priority
   - ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - ☐ Box No. IV Lack of unity of invention
   - ☒ Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - ☐ Box No. VI Certain documents cited
   - ☐ Box No. VII Certain defects in the international application
   - ☐ Box No. VIII Certain observations on the international application

Date of submission of the demand	Date of completion of this report
18.05.2004	07.02.2005
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Form PCT/IPEA/409 (cover sheet) (January 2004)

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/SE2002/001903

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ This report is based on a translation from the original language into the following language _____, which is the language of a translation furnished for the purposes of:

☐ international search (under Rules 12.3 and 23.1(b))
☐ publication of the international application (under Rule 12.4)
☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report):*

☐ the international application as originally filed/furnished

☒ the description:

pages 1-15 as originally filed/furnished
pages* _____ received by this Authority on _____
pages* _____ received by this Authority on _____

☒ the claims:

pages _____ as originally filed/furnished
pages* _____ as amended (together with any statement) under Article 19
pages* 1-2 received by this Authority on 2005-02-02
pages* _____ received by this Authority on _____

☒ the drawings:

pages 1 as originally filed/furnished
pages* _____ received by this Authority on _____
pages* _____ received by this Authority on _____

☐ a sequence listing and/or any related table(s) – see Supplemental Box Relating to Sequence Listing.

3. ☐ The amendments have resulted in the cancellation of:

☐ the description, pages _____
☐ the claims, Nos. _____
☐ the drawings, sheets/figs _____
☐ the sequence listing *(specify)*: _____
☐ any table(s) related to the sequence listing *(specify)*: _____

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

☐ the description, pages _____
☐ the claims, Nos. _____
☐ the drawings, sheets/figs _____
☐ the sequence listing *(specify)*: _____
☐ any table(s) related to the sequence listing *(specify)*: _____

* *If item 4 applies, some or all of those sheets may be marked "superseded."*

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/SE2002/001903

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application

☐ claims Nos. _____

because:

☒ the said international application, or the said claims Nos. 8, 9
relate to the following subject matter which does not require an international preliminary examination *(specify)*:

See PCT Rule 67.1.(iv).: Methods for treatment of the human or animal body by surgery or therapy, as well as diagnostic methods.

It is not clear that the methods are performed in vitro.

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. _____
are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. _____ are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for said claims Nos. _____

☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form
- ☐ has not been furnished
- ☐ does not comply with the standard

the computer readable form
- ☐ has not been furnished
- ☐ does not comply with the standard

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in the Annex C-*bis* of the Administrative Instructions.

☐ See Supplemental Box for further details.

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/SE2002/001903

Box No. V **Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	1-7	YES
	Claims		NO
Inventive step (IS)	Claims	1-7	YES
	Claims		NO
Industrial applicability (IA)	Claims	1-7	YES
	Claims		NO

2. Citations and explanations (Rule 70.7)

Reference is made to the following documents:

D1: WO 9400139 A1 (BIOGAIA BIOLOGICS AB), 6 January 1994 (06.01.94), page 8, line 21 - line 36; page 12, line 15 - line 23, abstract

D2: Lactic acid bacteria: microbiology and functional aspects, Volume, 1998, Iván A. Casas et al, "Lactobacillus reuteri: An Effective Probiotic for Poultry and Oter Animals" page 508 - page 509

D3: Eukaryot Microbiol, vol. 46, no. 5, Sept-Oct 1999, Waters WR et al: Effects of Lactobacillus reuteri On Cryptosporidium parvum infection of gnotobiotic TCR-alpha-deficient mice", page 1, abstract, URL:http://www.nps.ars.usda.gov/publications/Publications.htm?SEQ-NO-115=106105

D4: WO 9917788 A1 (ABBOTT LABORATORIES), 15 April 1999 (15.04.99), abstract

The invention relates to the use of Lactobacillus reuteri strains as immune enhancing agents and methods for improving immune-functions in mammals using Lactobacillus reuteri strains in products thereof. The strains exhibit good toxin binding, a neutralising effect and good CD4+ cell recruitment.

.../...

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of: Box V

D1 describes a method of stimulating the immune system of poultry using Lactobacillus reuteri preparations. The preparations alter the animals' T-cells. On page 8, lines 33-36, D1 describes a method where a high number of mammalian homologies of CD-4 antigen-bearing T-cells appear in the ileum lamina propria tissue of L.reuteri-treated chicks. It is not clear from D1 that the composition gives good CD4+ cell recruitment.

D2 describes the effect of Lactobacillus reuteri colonisation on GUT-associated lymphoid tissue (GALT) in developing chickens. On page 508, D2 describes that L.reuteri-treated chicks have significantly more CD4+ (helper T cells) T cells.

The cited documents represent the general state of the art.

The invention defined in claims 1-7 is not disclosed by any of these documents.

The cited prior art does not give any indication that would lead a person skilled in the art to the claimed Lactobacillus reuteri strains that exhibit toxin binding, a neutralising effect and good CD4+ cell recruitment. Therefore, the claimed invention is not obvious to a person skilled in the art.

Accordingly, the invention defined in claims 1-7 is novel and is considered to involve an inventive step. The invention is industrially applicable.

Example 6. Biochemistry of blood

Blood samples were taken on day 0 and day 28, and were analyzed for hemoglobin, hematocrit, thrombocytes, luecocytes, C-reactive protein, potassium, sodium, creatinine, b-urea, p-glucose, cholesterol, HDL (high density lipoproteins), LDL (low density lipoproteins), VLDL (very low density lipoproteins), tricgycerides, total bilirubin, urate, ALAT, alkaline phosphatase and lactate.

Most blood tests were normal, both before and after intake of *L. reuteri*. There were a few outliers in the blood variables, but no clinically significant abnormalities were found and no systematic changes were observed following the treatment.

Example 7. DNA Fingerprinting

DNA fingerprinting analysis was performed on selected *L. reuteri* isolates from the study. Thus, fecal isolates from three subjects who had consumed *L. reuteri* for 28 days were taken as well as one isolate from a duodenal biopsy and one isolate from an ileal biopsy both taken on day 0 before *L. reuteri* administration. All isolates were found to have a 98% genetic similarity to each other. All of these isolates showed a 97% similarity to SD2112, the strain incorporated into the tablets.

Example 8: Formulation of product to improve immune-function in humans

In this example is *L. reuteri* SD2112, ATCC 55730 selected, using the methods above for toxin neutralization and CD4+ cell recruitment, in order to add to a standard yogurt. The *L. reuteri* strain is grown and lyophilized, using standard methods for growing *Lactobacillus* in the dairy industry. This culture are then added to previously fermented milk, using traditional yogurt cultures, at a level of 10E+7 CFU/gram of yogurt, and the yogurt is used by humans as a way to improve their immune function.

While the invention has been described with reference to specific embodiments, it will be appreciated that numerous variations, modifications, and embodiments are possible, and accordingly, all such variations, modifications, and embodiments are to be regarded as being within the spirit and scope of the invention.

CLAIMS

1. Use of Lactobacillus reuteri strains that
   a. exhibit good toxin binding and neutralizing effect; and
   b. exhibit good CD4+ cell recruitment

   for the production of a composition for improving immune-function in mammals.

2. A product comprising a strain with at least the characteristics according to claim 1.

3 The product of claim 2 wherein the product is formulated as a food containing cells of the selected strain.

4 The product of claim 2 wherein the product is formulated as a tablet containing cells of the selected strain.

5. The product of claim 2 wherein the product is formulated as a dietary supplement containing cells of the selected strain.

6. The product of claim 2 wherein the product is formulated as a confectionery containing cells of the selected strain.

7. The product of claim 2 wherein the product is formulated as a drug containing cells of the selected strain.

8. The use of the culture supernatant of *L.reuteri* ATCC 55730 for neutralizing bacterial Toxins.

9. A method for improving immune-function in mammals using *Lactobacillus reuteri* strains in products containing cells of such strains, comprising: using strains that
   a. exhibit good toxin binding and neutralizing effect;
   b. and exhibit good CD4+ cell recruitment.